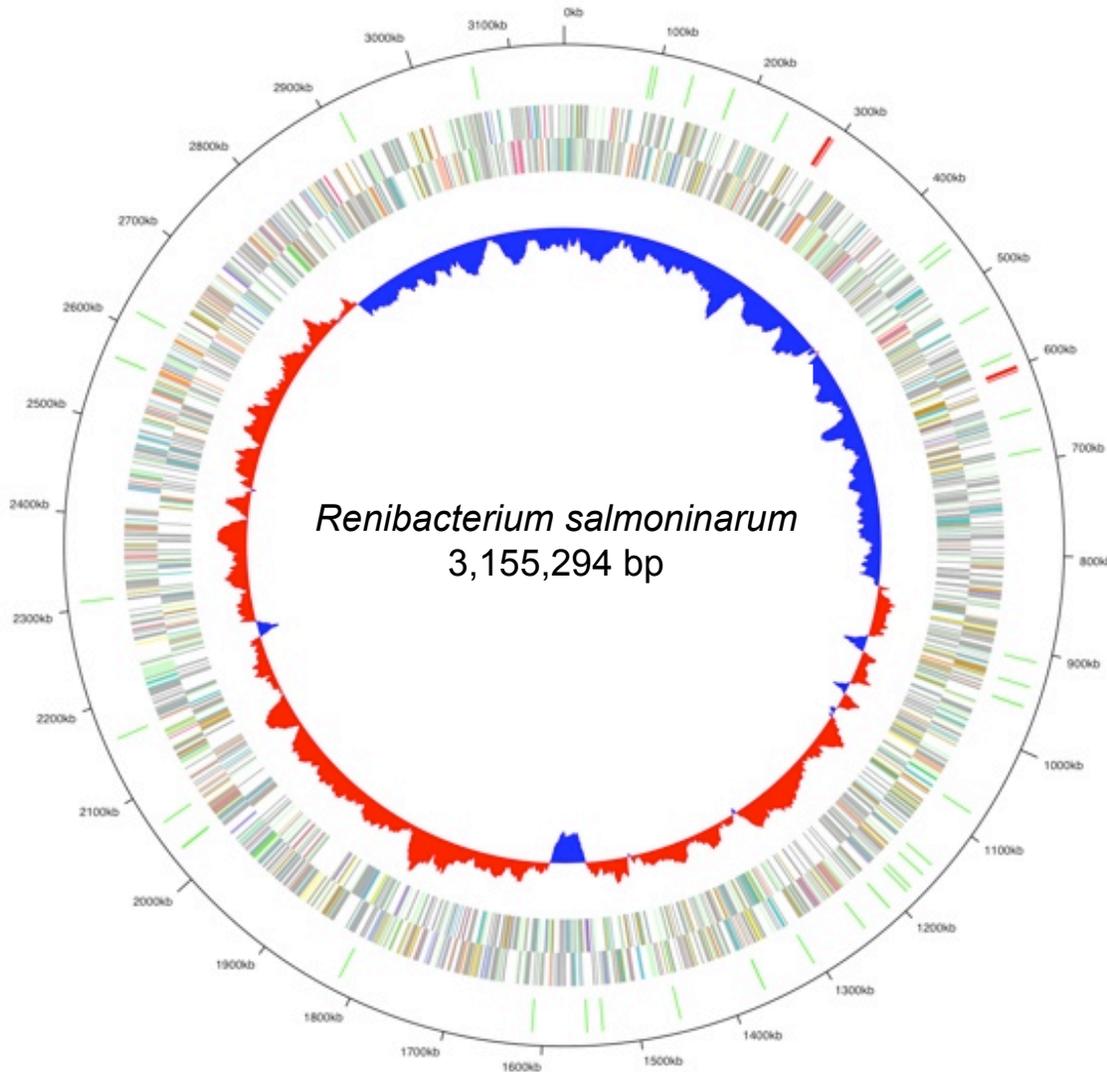


The *Renibacterium salmoninarum*
Genome Sequencing Project
First Findings: Overview of the Genome and
Examination of Antibiotic Resistance Genes
and Cell Surface Proteins

Mark S. Strom, Sudheesh Ponnerassary,
Samuel Crane, and Donald Chen
Northwest Fisheries Science Center, NOAA Fisheries



Single circular
chromosome

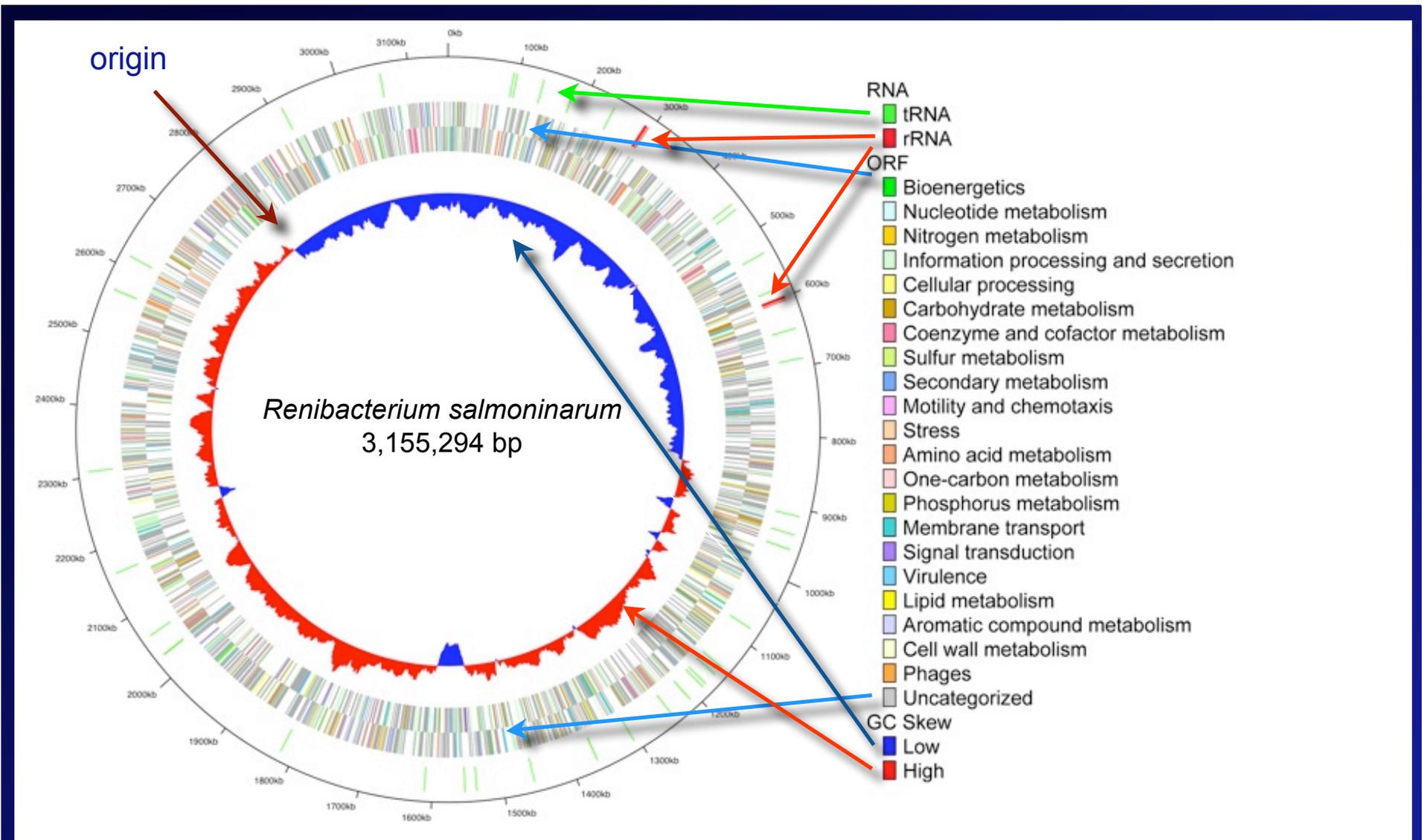
3,155,294 bp

56.27% G+C

3,667 ORFs

ERGO assigned
function to 2,333
(63.6%)

Manual annotation
increased assigned
functions to ~88%



Bioinformatics tools

ERGO

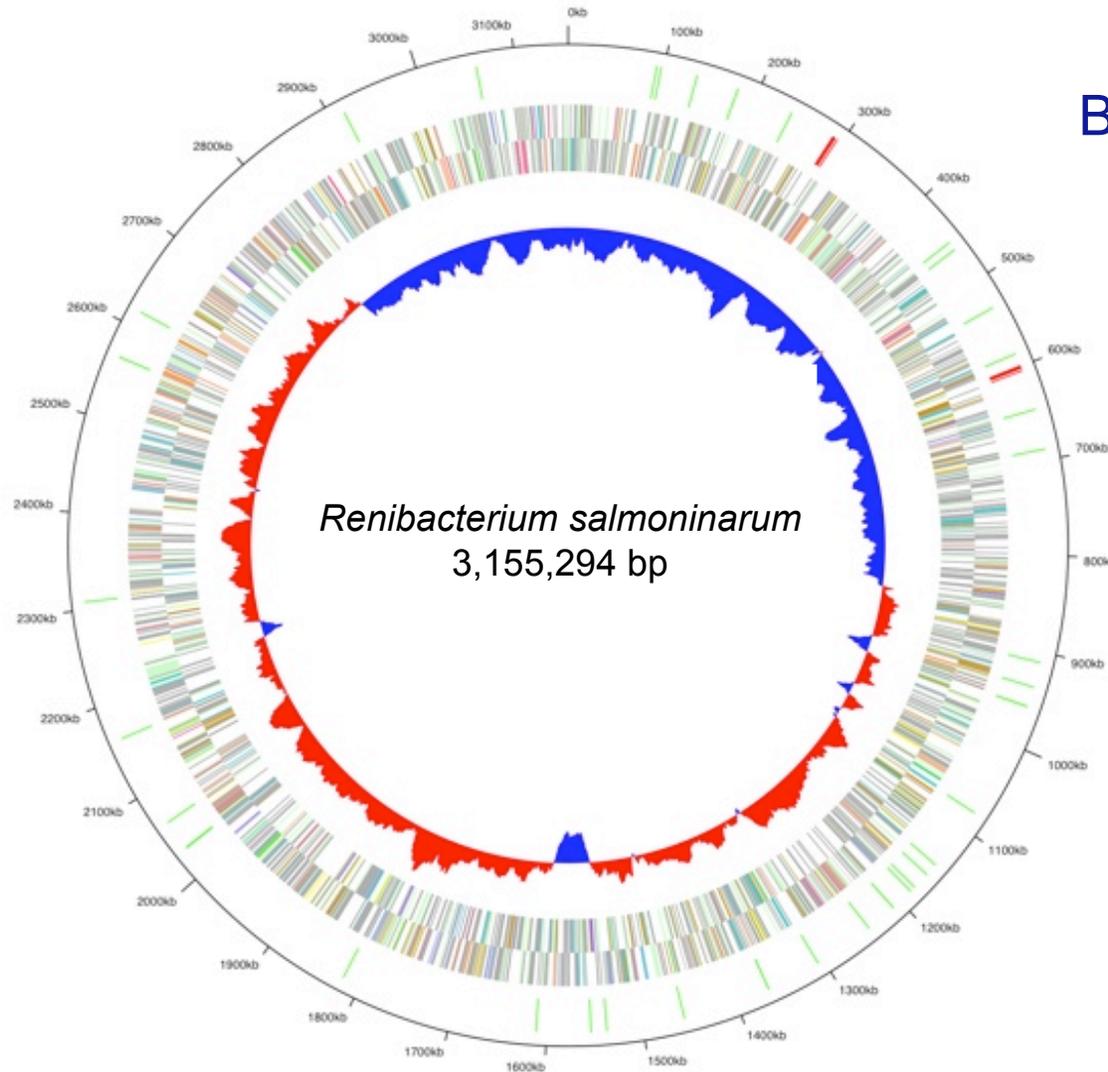
Pfam (protein families)

TMPred (transmembrane predictions)

SignalP (signal peptide search)

COGs (clusters of orthologous groups)

BLAST (sequence similarity)

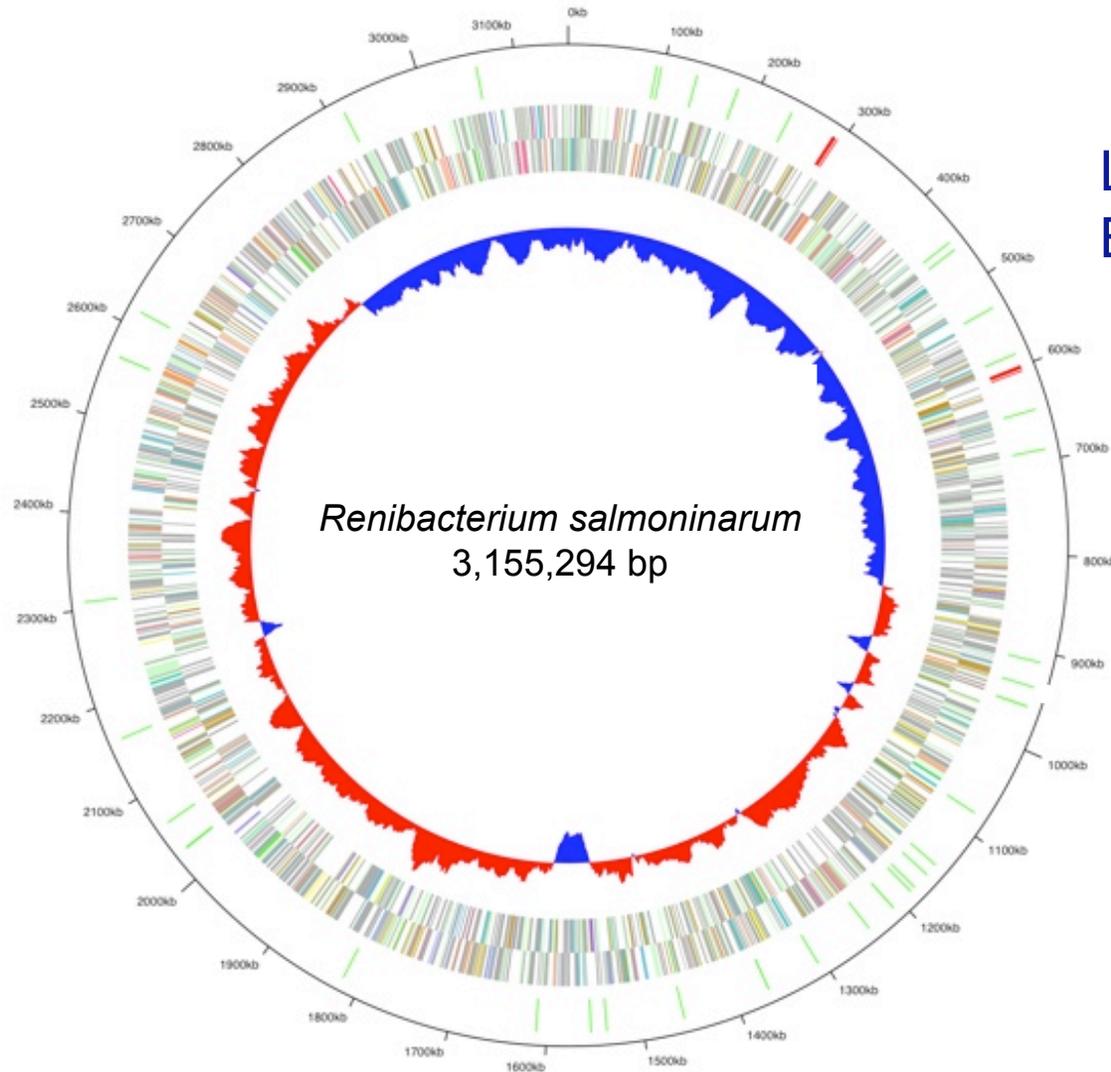


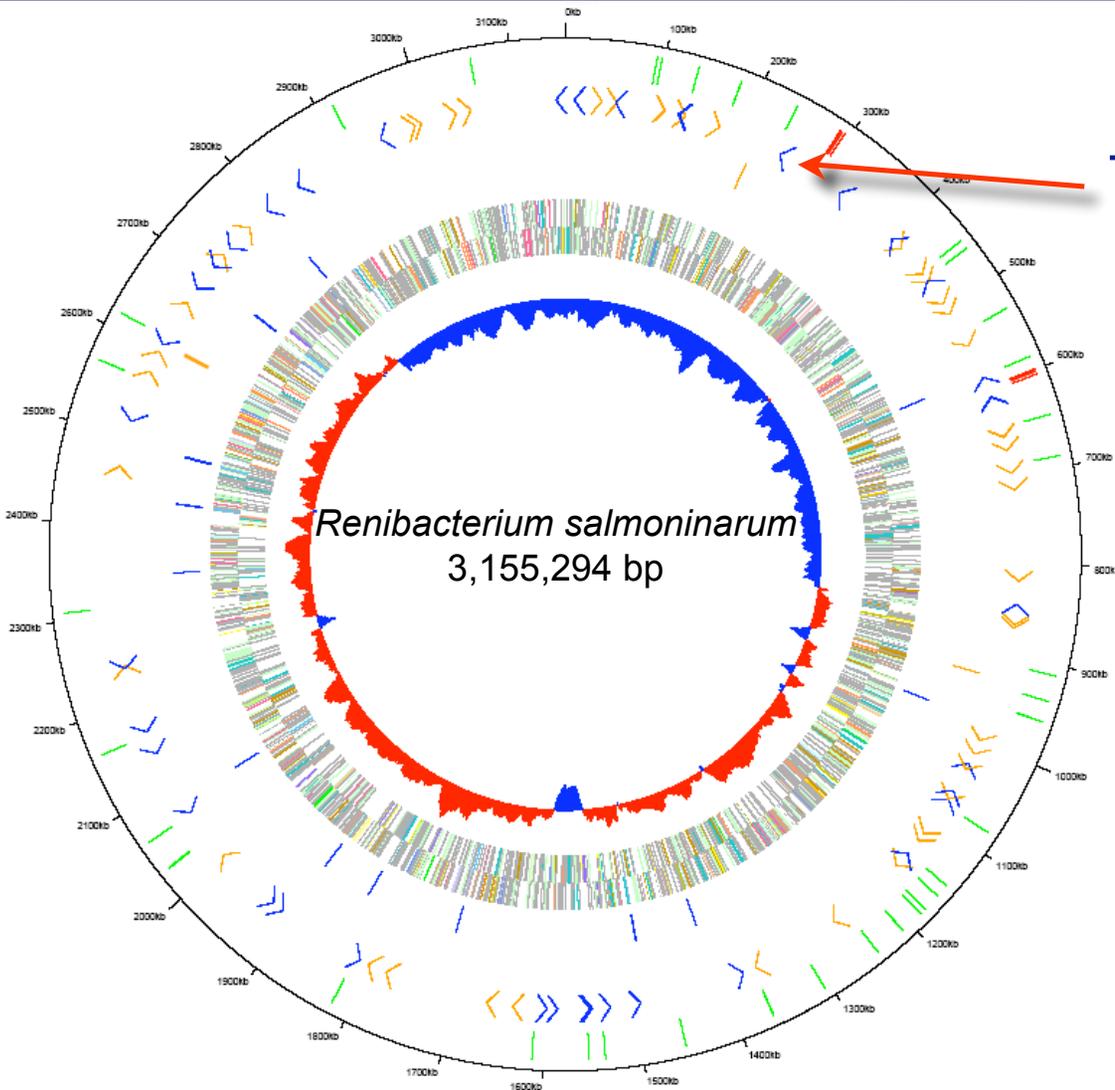
Genome Comparisons

	Genomic DNA (mbp)	Coding DNA (mbp)	% Coding	% GC
<i>Renibacterium salmoninarum</i> 33209	3.16	2.87	90.8	56.3
<i>Corynebacterium diphtheriae</i> NCTC 13129	2.49	2.24	90.0	53.5
<i>Streptomyces coelicolor</i> A3(2)	8.67	7.94	91.6	72
<i>Escherichia coli</i> K12	4.64	4.13	89.0	50.8

Lipid and Amino Acid Biosynthesis

manually examined and found essentially complete with all major components represented



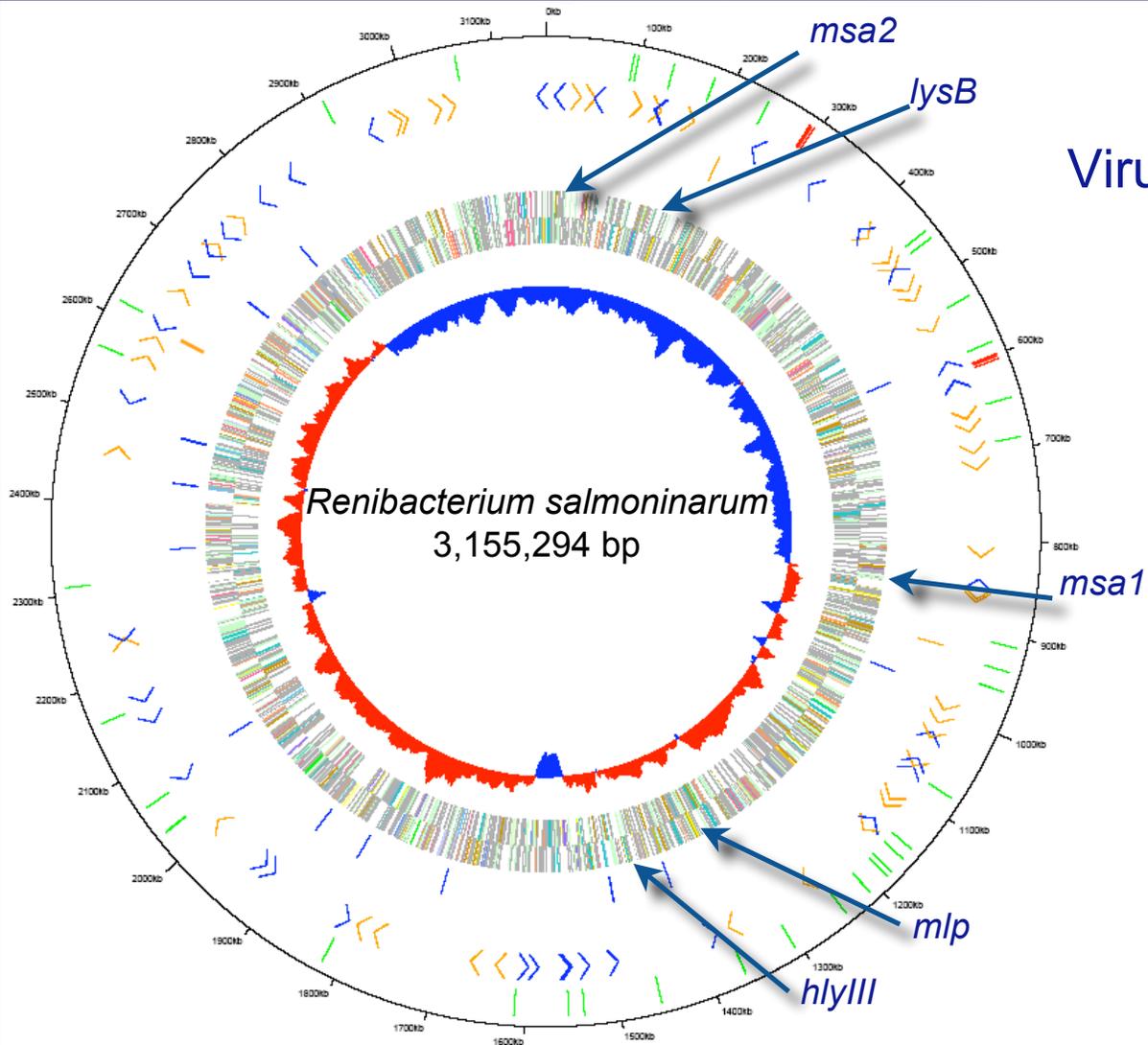


Transposases

78 insertion elements/transposable elements

- 69 copies of IS994
- 9 copies of RsIS2
- no “hot spot” for insertion preference
- responsible for large number of pseudogenes, gene duplication, deletion, genome rearrangement

Virulence Genes



bacterial infection treatment options

- antibiotics
 - relatively inexpensive and often very effective
 - but selective pressure for resistance development, environmental issues

Antibiotics in fish culture

- tetracycline
- sulfonamides
- quinolones
- amoxicillin
- florfenicol
- macrolides (erythromycin)

use of antibiotics to treat BKD

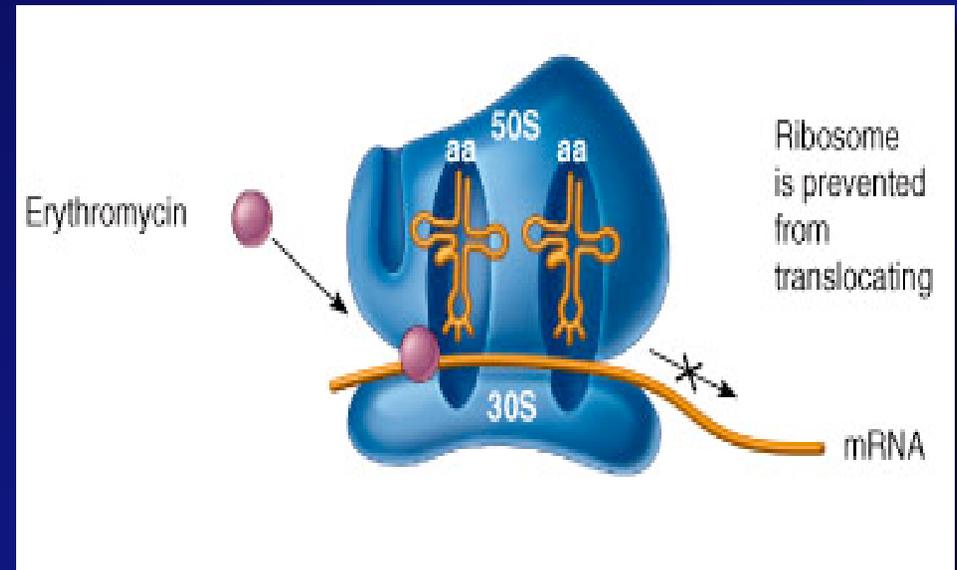
- no approved antibiotic
- erythromycin most common
- subject of FDA INAD (investigational new animal drug) permit process
- azithromycin used in ESA-listed stocks, captive rearing programs

macrolides and BKD treatment

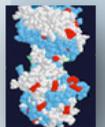
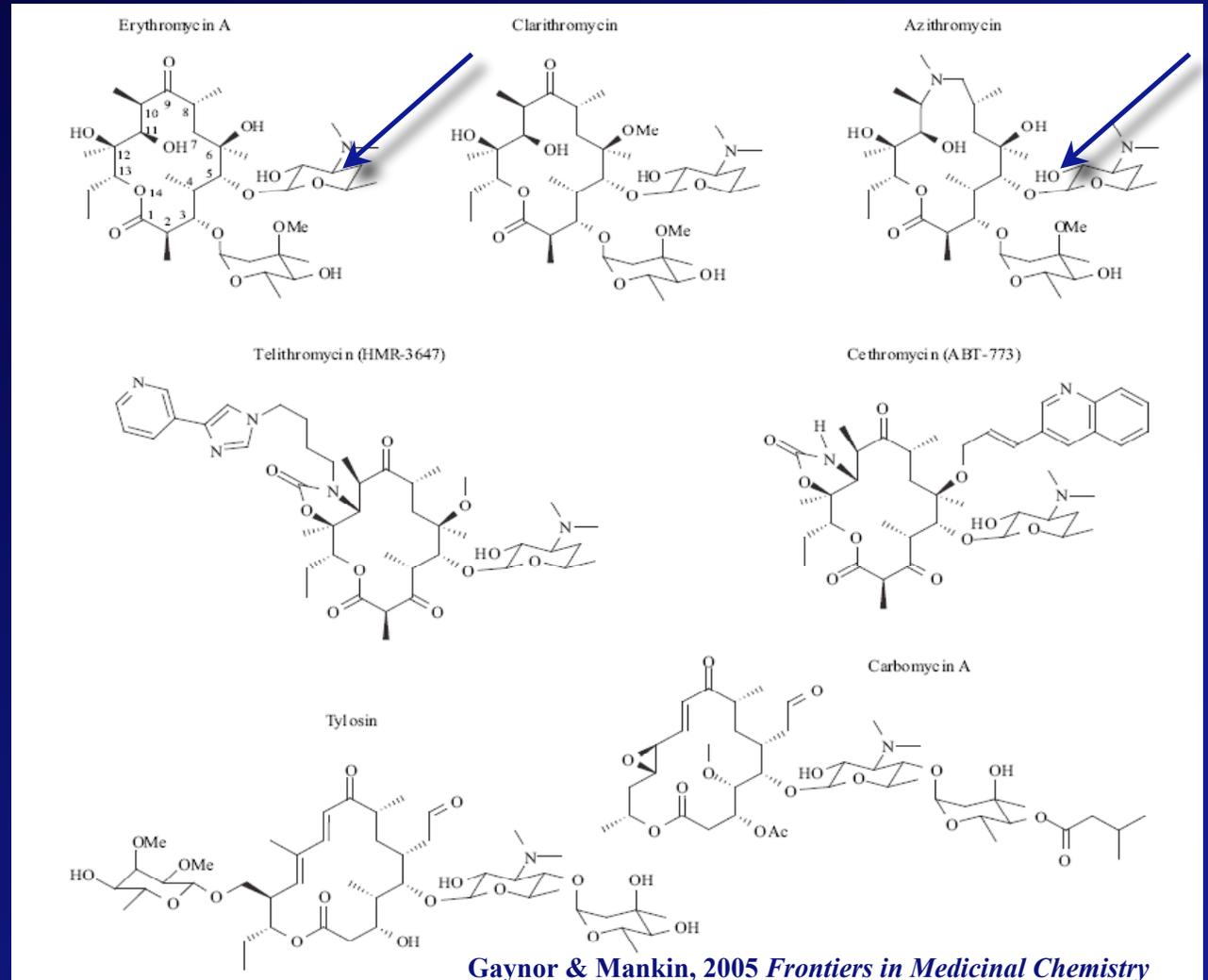
- Erythromycin
 - MIC < 0.62 to 5.47 $\mu\text{g/ml}$ (Bandin *et al*); 0.1 $\mu\text{g/ml}$ in KDM2 broth
 - medicated feed - 40 to 100 mg/Kg for 28 days
 - adult broodstock injection (40 mg/Kg IP), transmitted to eggs, not transmitted to progeny
- Azithromycin
 - MIC 0.0125 $\mu\text{g/ml}$
 - medicated feed - 20 mg/Kg for 14 days
 - adult broodstock injection (40 mg/Kg IP), transmitted to eggs, transmitted to progeny and persists for several weeks

macrolide mode of action

- Inhibition of the progression of the nascent peptide chain during early rounds of translation
- Promotion of peptidyl tRNA dissociation from the ribosome
- Inhibition of peptide bond formation
- Interference with 50S subunit assembly



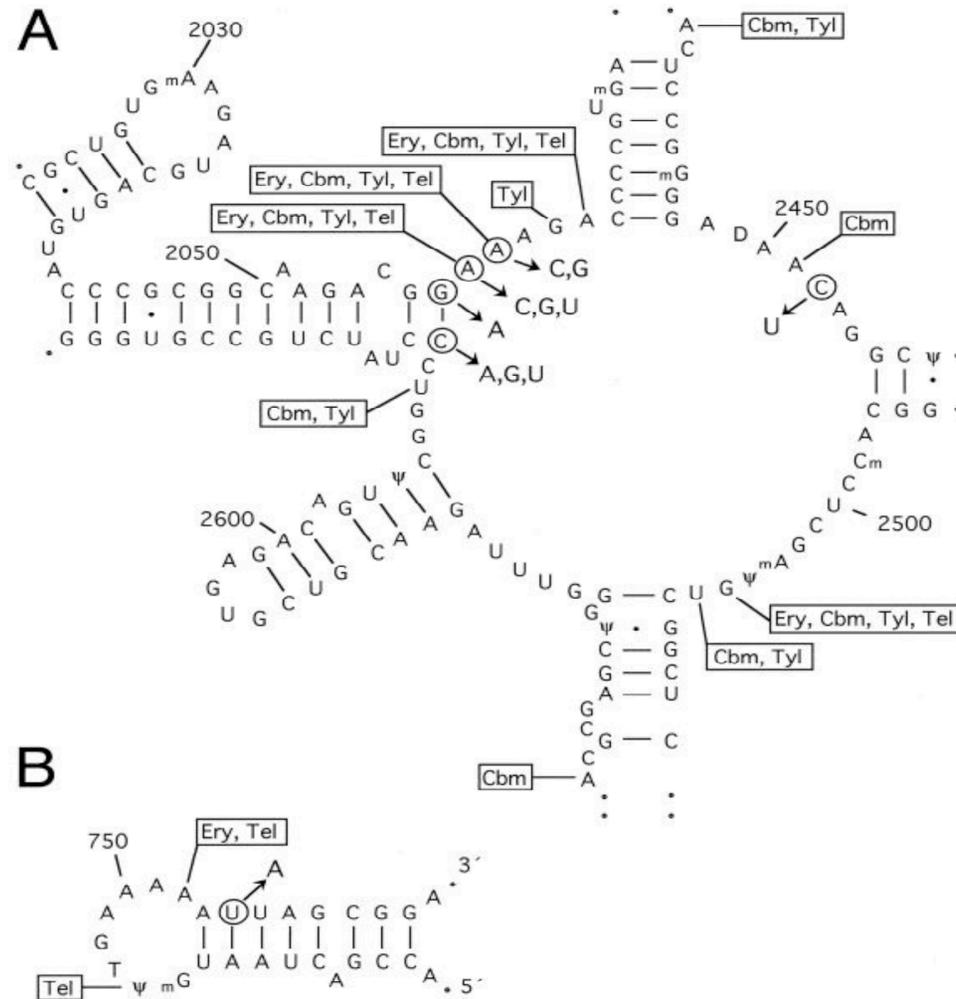
macrolide antibiotics



possible macrolide resistance encoding genes in *R. salmoninarum*

- macrolide efflux proteins and transporters
 - proton/ ATP activated pumps (mef)
- ribosomal (drug target) modification
 - rRNA methylation (erythromycin resistance methylases)
 - rRNA mutations
 - ribosomal protein mutations (L4, L22)
- macrolide inactivation
 - phosphorylation, glycosylation, cleavage of lactone ring(erythromycin esterase)

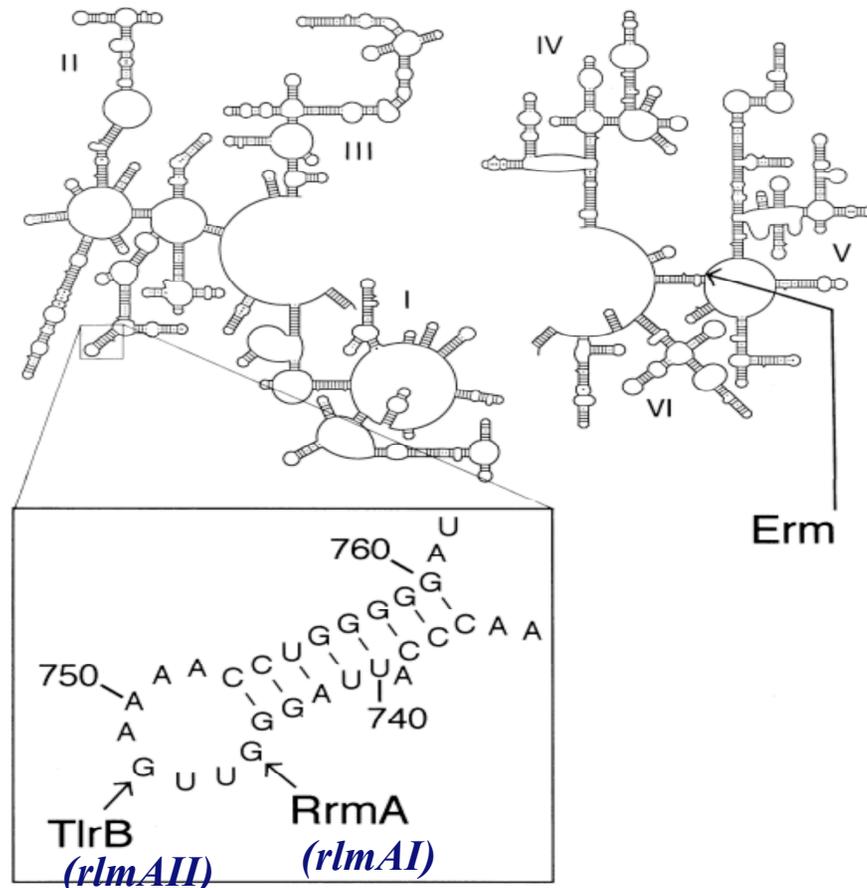
Target nucleotides on 23s rRNA



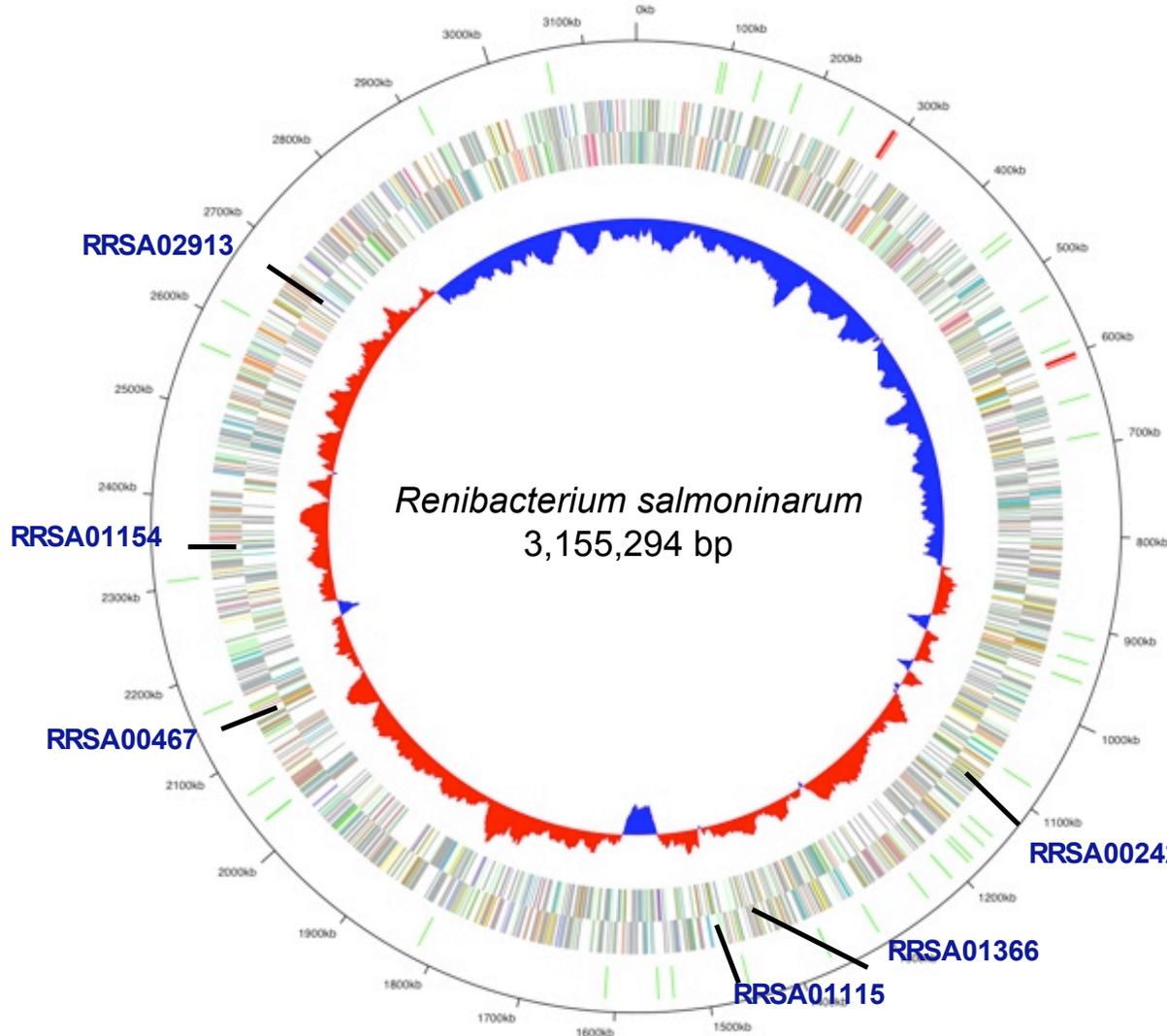
Vester and Douthwaite, 2001 *Antimicrob. Agents Chemother.*



Action of *rlm* and *erm* genes



Gaynor & Mankin, 2005 *Frontiers in Medicinal Chemistry*



Genes encoding potential macrolide resistance factors

23S rRNA m(1)G 745 methyltransferase (*rlmAII*)
RRSA01154

Macrolide-efflux protein
RRSA00467

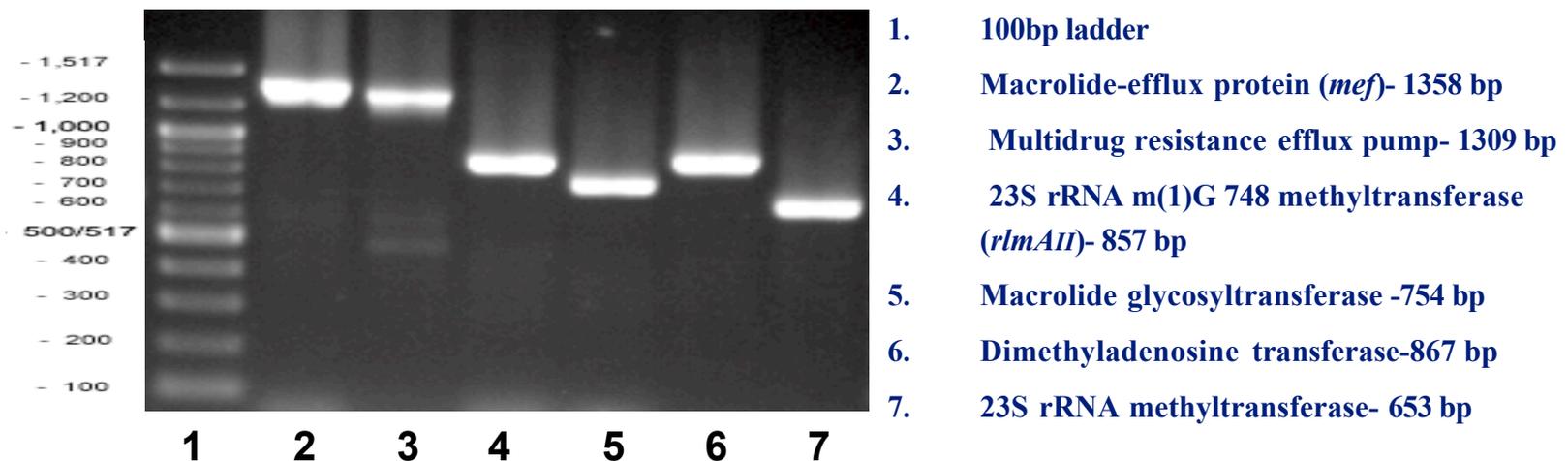
Multidrug resistance efflux pump
RRSA02913

23S rRNA methyltransferase
RRSA00242

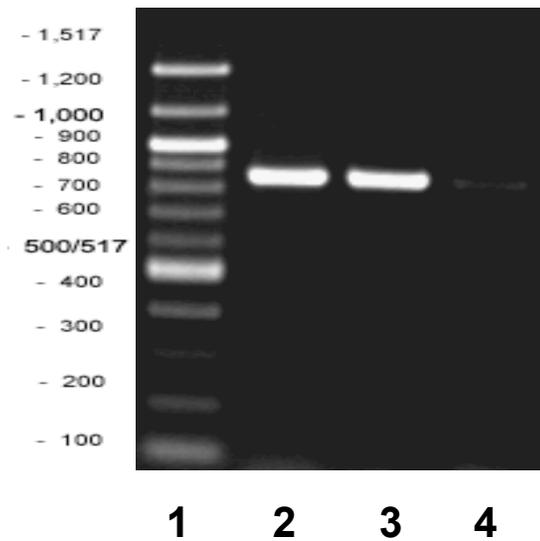
Macrolide glycosyltransferase
RRSA01115

Dimethyladenosine transferase
RRSA01366

Macrolide antibiotic resistance genes amplified from genomic DNA of *R. salmoninarum* 33209



rlmA_{II} gene expression in *R. salmoninarum* 33209 exposed to 0.0625 µg/mL Erythromycin

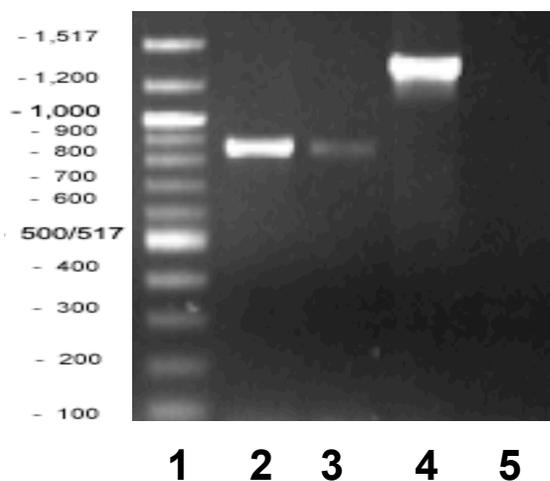


1. 100bp marker
2. PCR product
3. RT-PCR product-induced
4. RT-PCR product - uninduced

Similarity of *Rs* RImA with other species RImA

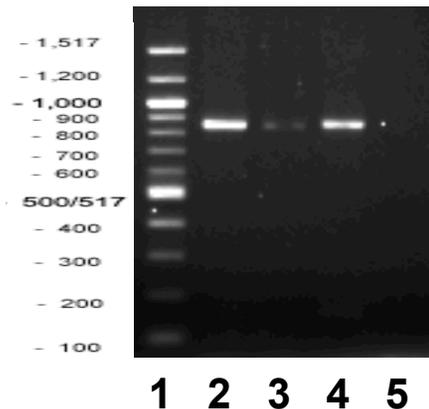
Species	aa length	Species	aa length	% Similarity
<i>Streptomyces fradiae</i>	280	<i>R. salmoninarum</i>	300	33
<i>Corynebacterium glutamicum</i>	283	<i>R. salmoninarum</i>	300	32
<i>Acinetobacter sp.</i>	275	<i>R. salmoninarum</i>	300	28
<i>Yersinia pseudotuberculosis</i>	279	<i>R. salmoninarum</i>	300	28
<i>Escherichia coli</i>	269	<i>R. salmoninarum</i>	300	27
<i>Salmonella enterica</i>	269	<i>R. salmoninarum</i>	300	25
<i>Salmonella typhimurium LT2</i>	269	<i>R. salmoninarum</i>	300	24
<i>Streptococcus pneumoniae</i>	282	<i>R. salmoninarum</i>	300	23

rlmAII and *mef* gene expression in *R. salmoninarum* 33209 exposed to 1 $\mu\text{g}/\text{mL}$ Erythromycin



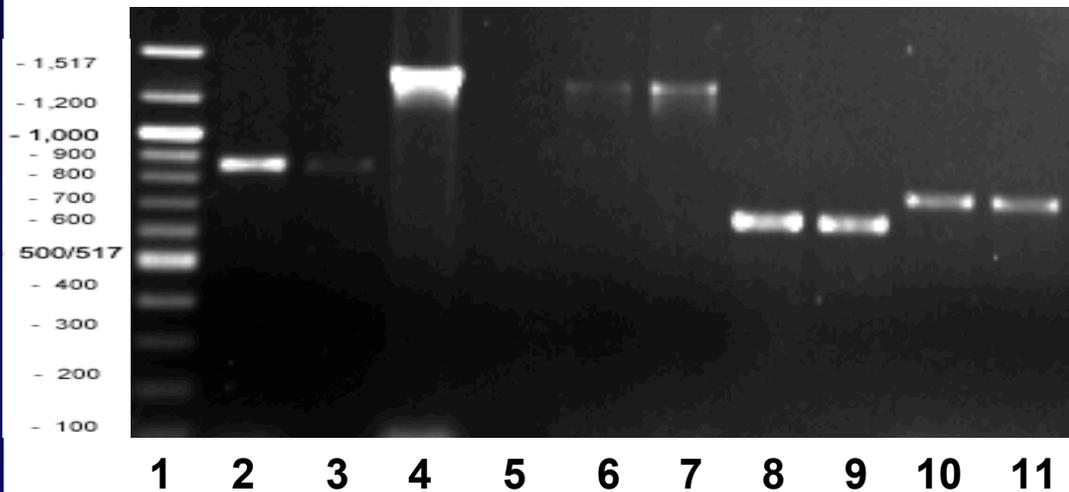
1. 100bp marker
2. *rlmAII* RT-PCR product -induced
3. *rlmAII* RT-PCR product -uninduced
4. *mef* RT-PCR product -induced
5. *mef* RT-PCR product -uninduced

**rlmAII and Dimethyl adenosine transferase gene
expression in *R. salmoninarum* clinical isolate (from
chinook salmon) after growth in 0.1 µg/mL
Azithromycin**



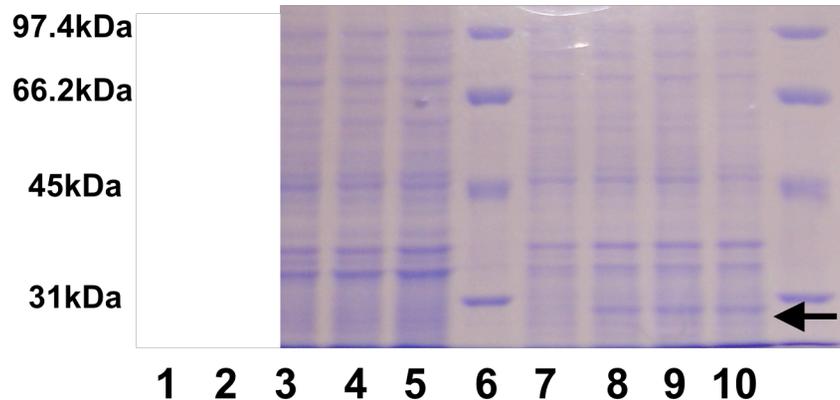
1. 100bp marker
2. *rlmAII* RT-PCR product -induced
3. *rlmAII* RT-PCR product -uninduced
4. DMAT RT-PCR product -induced
5. DMAT RT-PCR product -uninduced

Expression pattern of macrolide resistance genes in *R. salmoninarum* 33209 exposed to 1 $\mu\text{g}/\text{mL}$ Erythromycin

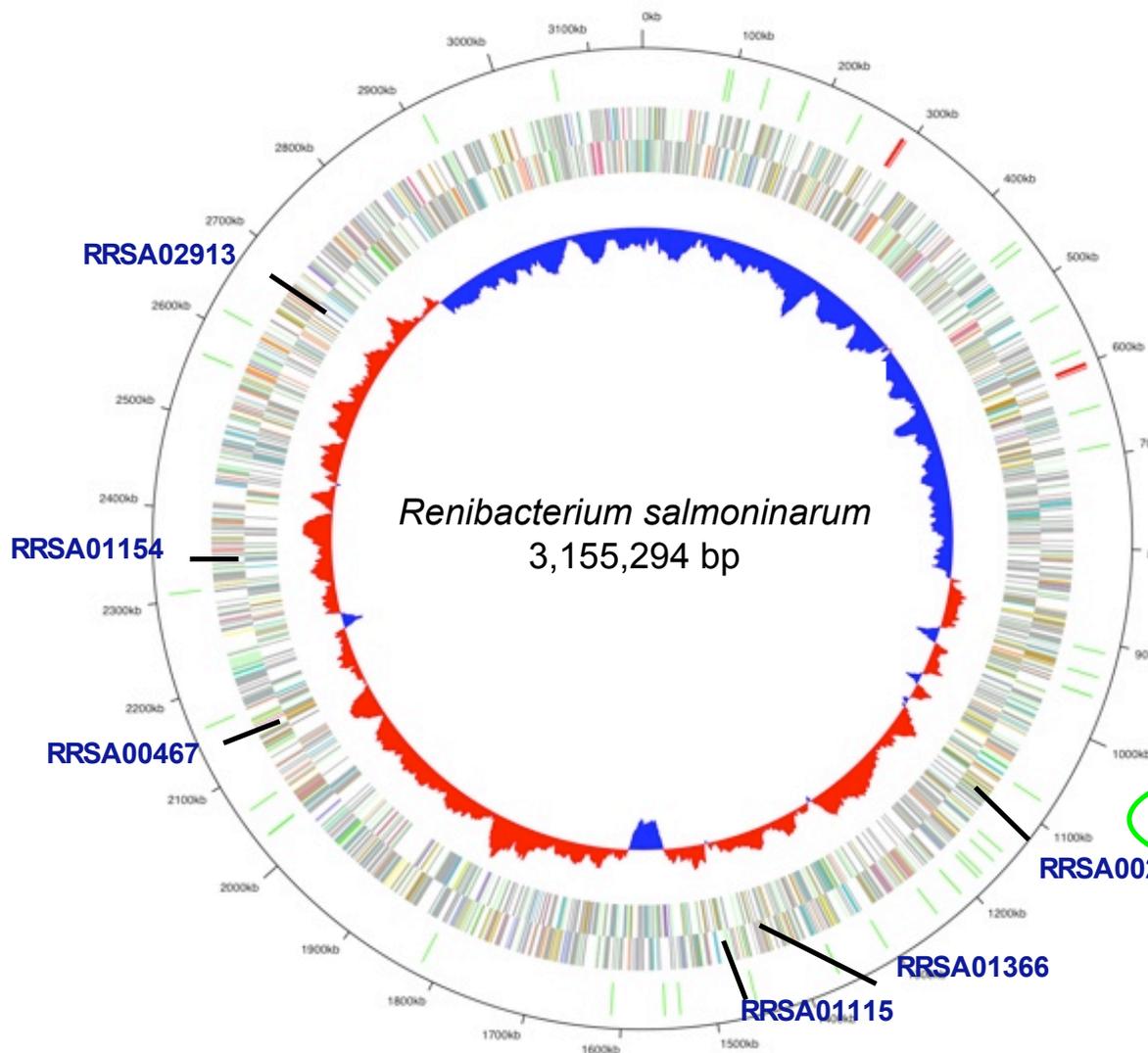


1. 100bp ladder
2. 23S rRNA m(1)G 748 methyltransferase (*rlmAII*) -I
3. 23S rRNA m(1)G 748 methyltransferase (*rlmAII*)- UI
4. Macrolide-efflux protein (*mef*)- I
5. Macrolide-efflux protein (*mef*)- UI
6. Multidrug resistance efflux pump- I
7. Multidrug resistance efflux pump- UI
8. 23S rRNA methyltransferase- I
9. 23S rRNA methyltransferase- UI
10. Macrolide glycosyltransferase- I
11. Macrolide glycosyltransferase- UI

Recombinant RImA_{II} expression in *E. coli*



1. Marker
 2. Uninduced- 0 hr
 3. Uninduced- 1 hr
 4. Uninduced- 2 hr
 5. Uninduced- 4 hr
 6. Marker
 7. *Induced- 0 hr
 8. Induced- 1 hr
 9. Induced- 2 hr
 10. Induced- 4 hr
- * Induced with 1.0 mM IPTG



Genes encoding potential macrolide resistance factors

23S rRNA m(1)G 745 methyltransferase (*rlmAII*)
RRSA01154

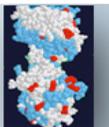
Macrolide-efflux protein
RRSA00467

Multidrug resistance efflux pump
RRSA02913

23S rRNA methyltransferase
RRSA00242

Macrolide glycosyltransferase
RRSA01115

Dimethyladenosine transferase
RRSA01366



further macrolide studies

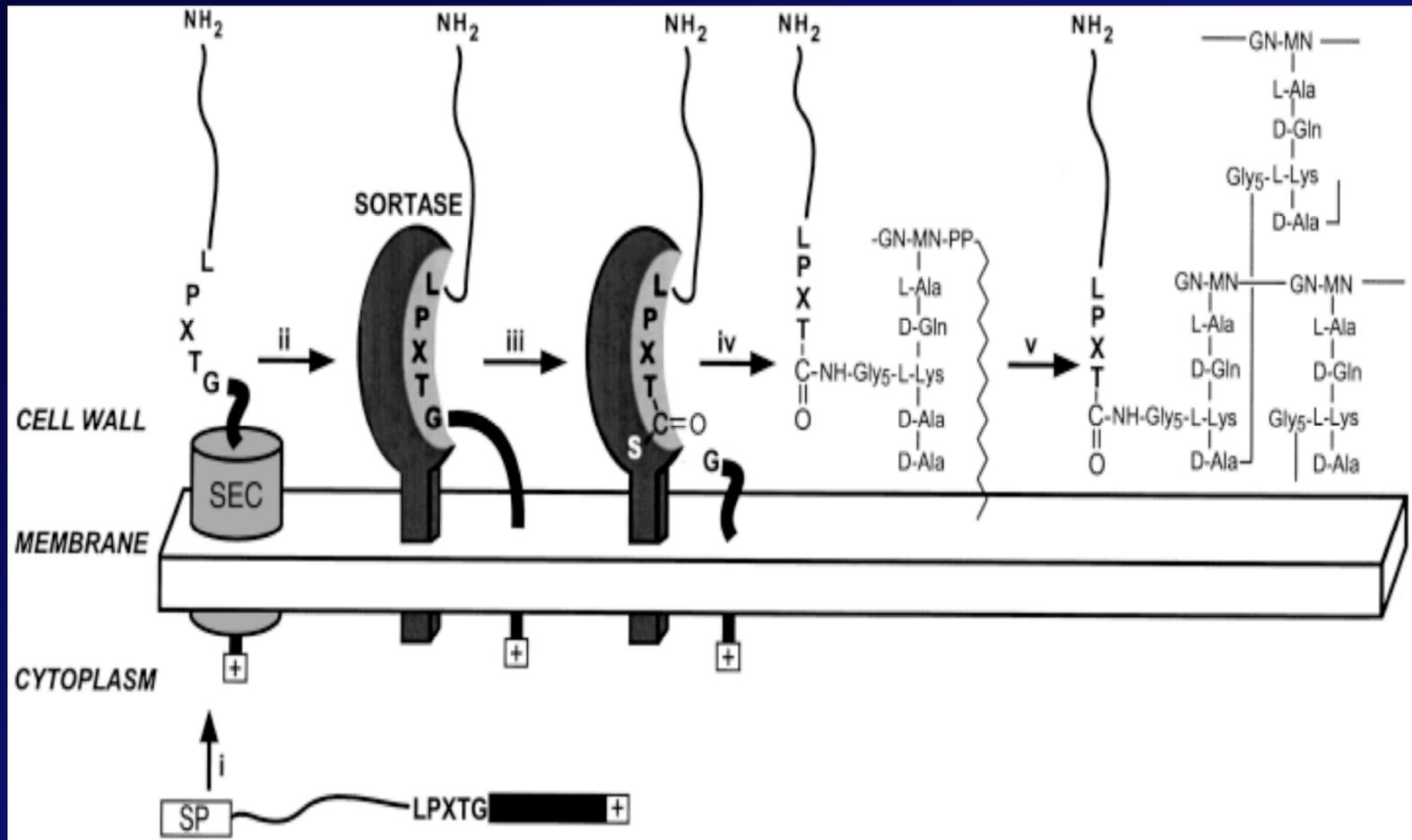
- Determine macrolide gene expression with increasing erythromycin concentrations
- Stability of resistance
- Analyze macrolide resistance gene expression pattern in different clinical isolates from fish untreated vs treated with macrolide drugs using cDNA microarrays or qRT-PCR
- Determine correlation between macrolide resistance gene expression pattern and drug efficacy in treating BKD

bacterial infection treatment options

- antibiotics
 - relatively inexpensive and often very effective
 - but selective pressure for resistance development, environmental issues
- vaccines - rational antigen selection
- anti-infectives
 - novel approach
 - reduction in virulence
 - no selective pressure

gram positive sortases

- mediate anchoring of surface proteins in Gram + bacteria
 - e.g., fibronectin or collagen binding proteins, invasins, adhesins, internalins

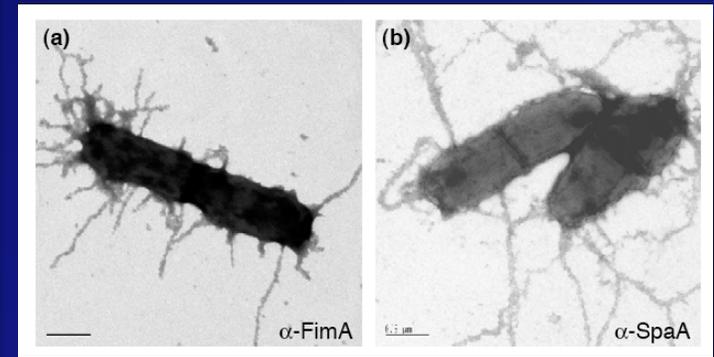
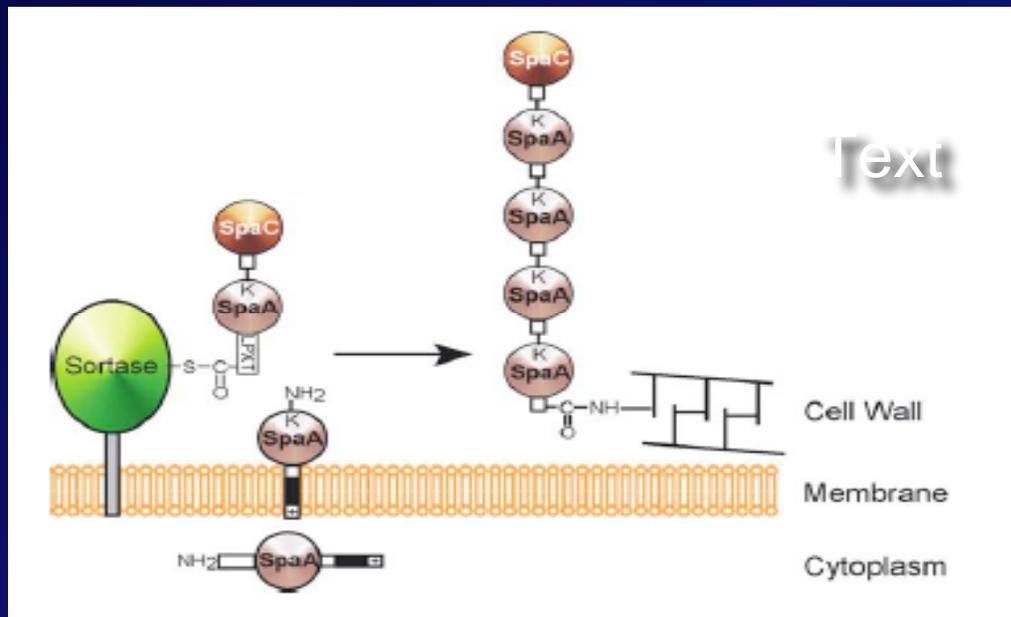


gram positive sortases

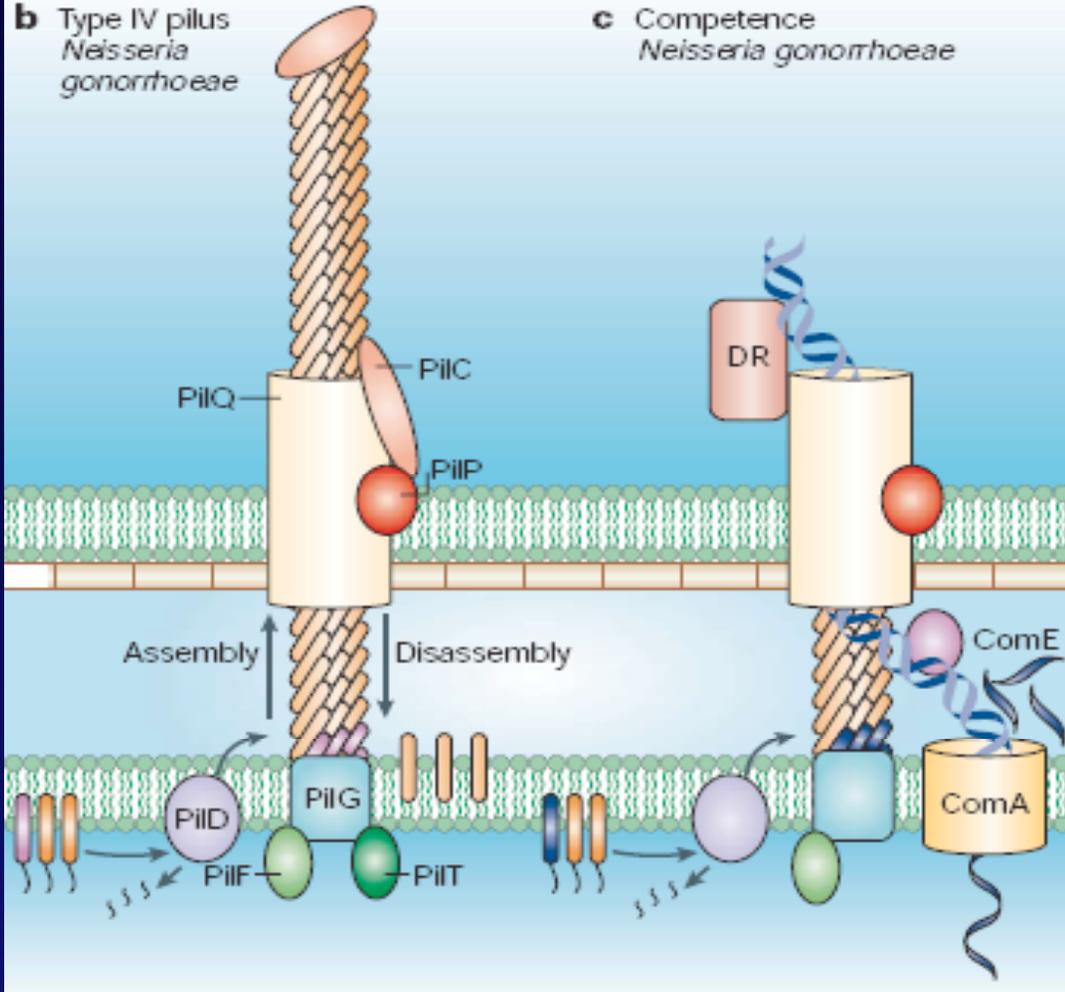
- inactivation or interference in sortase activity attenuates virulence
 - number of small molecule inhibitors already demonstrated for other G+ sortases

Sortase mediated pilin formation in Gram positive bacteria

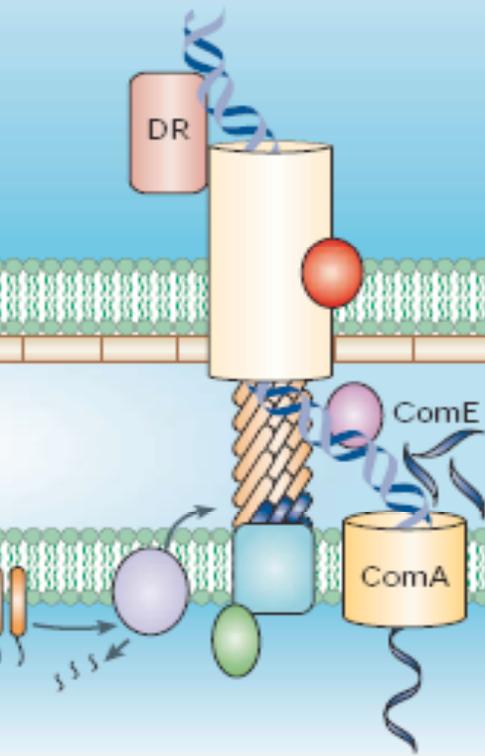
Actinomyces naeslundii
Corynebacterium diphtheriae
Streptococcus parasanguis
*Ruminococcus albus**



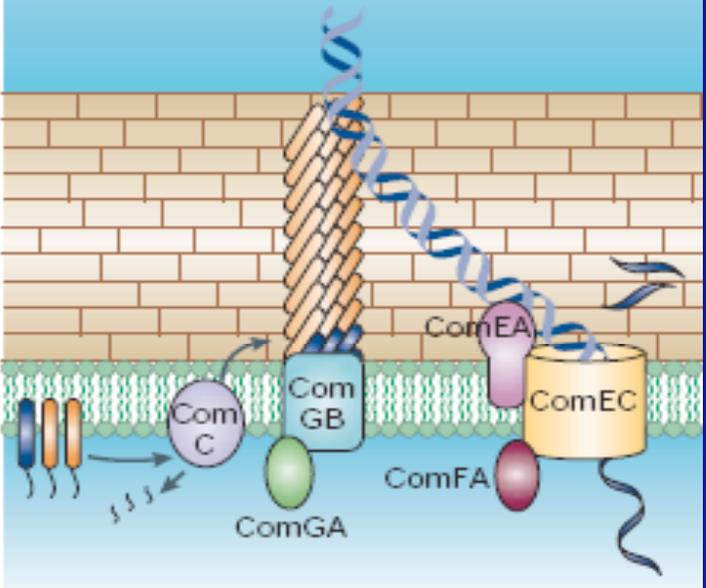
b Type IV pilus
Neisseria gonorrhoeae



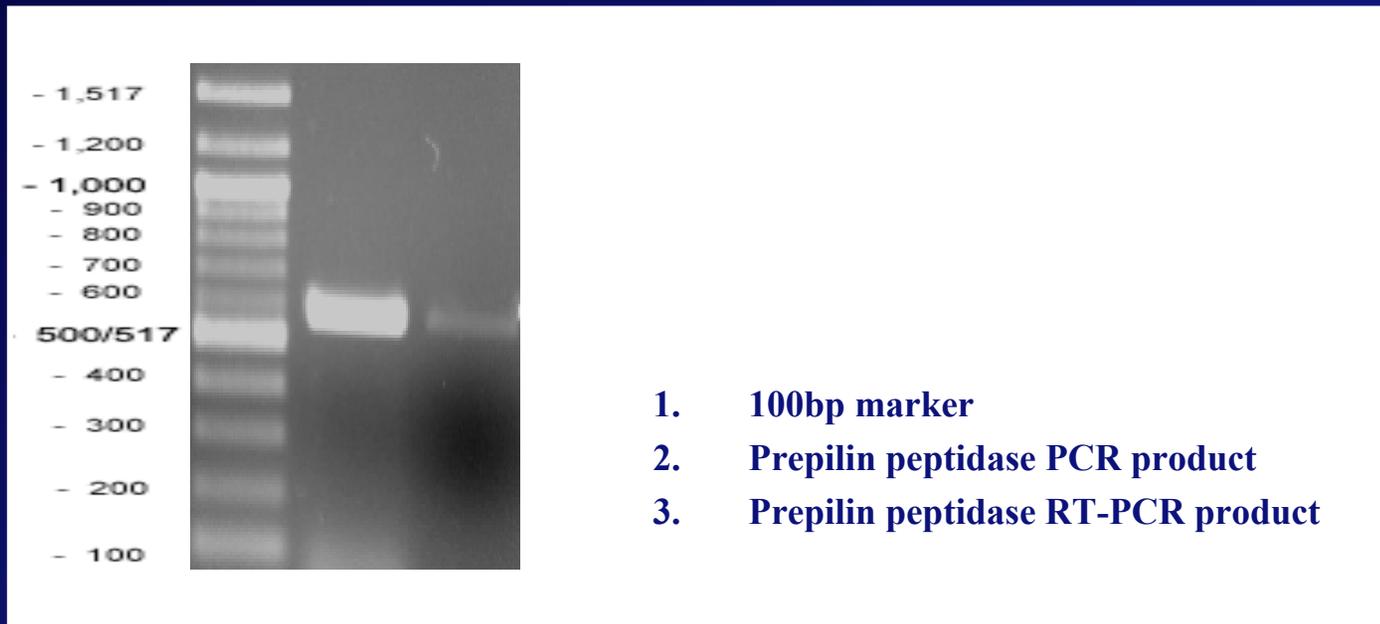
c Competence
Neisseria gonorrhoeae



d Competence
Bacillus subtilis



Prepilin peptidase gene expression in *R. salmoninarum* 33209



acknowledgements

Genome project

Greg Wiens - USDA NCCCWA

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